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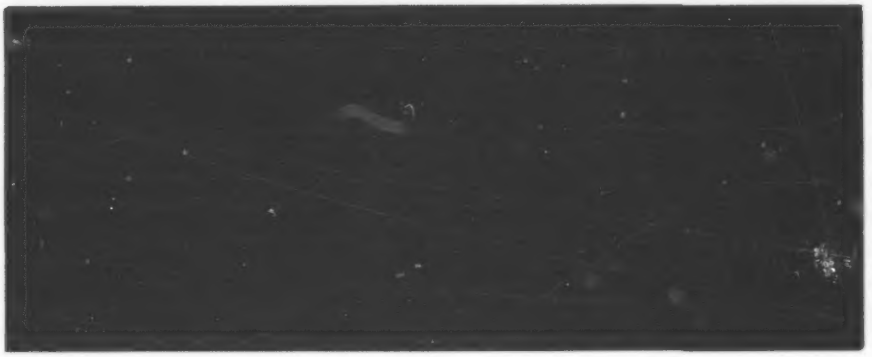

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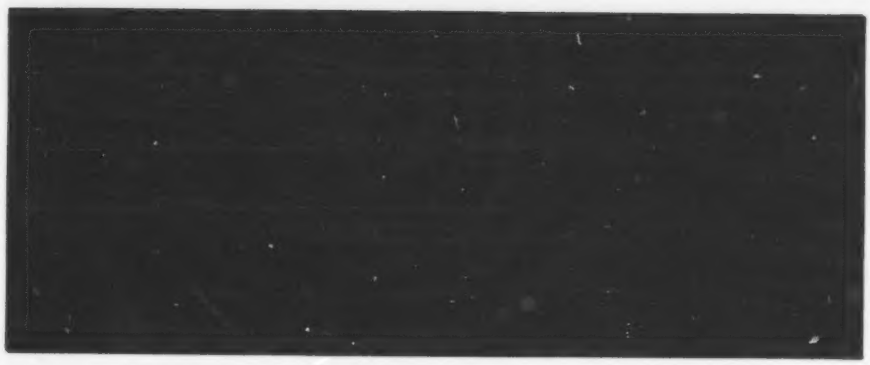

Technical/Final Report
to
Saskatchewan Agriculture Research Fund

Improvement of Production Efficiency of Livestock by Vaccination Against Somatostatin

S-86-01-0036
S-85-01-0015/5-85-26

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91-01755/

Not for public release

Somatostatin-immunization experiment - 16 (SRIF-16) in rats

Vaccines prepared: August 31, 1987
Primary injection: September 3, 1987
Booster injection: September 17, 1987

Animals: Sprague Dawley rats from Charles River

Objective:

To evaluate the dimyristoyl-lysine-somatostatin (SRIF) derivative as an immunogenic entity in association with dimyristoyl-lysine micelles (5% molar expression) or in association with the Ribi Adjuvant System (RAS). RAS consists of squalene oil droplets with monophosphoryl lipid A (MPL) as a B-cell mitogen. In addition, the micelle treatment was either retained at the injection site by use of Alhydrogel adjuvant or not by use of saline as vehicle. All treatments (4) contained equal amounts (50 ug) of somatostatin derivative, MPL and stearoyl muramyl dipeptide (MDP).

Treatments:

Control: injections of saline only; 0.4 ml/rat S.C. in two sites.

Yellow: Ribi Adjuvant System (RAS).

- To one vial of RAS add 0.5 ml chloroform and mix. Add 250/ug MDP-stearoyl in 0.25 ml chloroform:methanol (4:1) (C:M)
  Add 250 ug dimyristoyl lysine-SRIF in 0.12 ml DMF
- Evaporate solvents to dryness under N_2
- Add 2 ml saline and vortex
- inject 0.4 ml/rat S.C. in two sites

Orange: RAS.

- Warm one vial of RAS to 60°C
- Add 2.0 ml phosphate buffered saline (PBS) 0.05 M, pH 7.4 and vortex
- Continue vortexing and add:
  1. Stearoyl MDP, 250 ug in 0.25 ml C:M.
  2. Dimyristoyl-lysine-SRIF in 0.12 ml DMF.
- Continue vortexing, maintain at 60°C.
- Inject 0.4 ml/rat S.C. in two sites.

Green: Dimyristoyl-lysine-SRIF micelles in Alhydrogel.

The following compounds were used:

1. Dimyristoyl lysine-SRIF 250 ug in 0.12 ml DMF.
2. Dimyristoyl-lysine 1750 ug in 0.45 ml C:M.
3. Stearoyl-MDP, 250 ug in 0.25 ml C:M.
4. Monophosphoryl Lipid A (MPL) 250 ug in 0.5 ml C:M.

After vortexing the solvent is evaporated at 60°C under N_2. Reaction conducted in 16 mm test tube. To the lipid film add 2 ml saline and vortex repeatedly. Add 2 ml of Alhydrogel and vortex. Inject 0.8 ml/rat S.C. in two sites.

Blue: Dimyristoyl-lysine-SRIF micelles in saline.

Method similar to green, but Alhydrogel is not added.
Inject 0.4 ml/rat S.C. in two sites.

Materials: The dimyristoyl-lysine was supplied by Philom Bios. It was synthesized on to SRIF by peptide synthesis at the Alberta Peptide Institute. Incorporation of the dimyristoyl-lysine was judged to be 98% complete through amino acid analysis.

Results and discussion:

The immunization with micelles in saline (blue) gave significantly higher final absolute weights (Table 1), final cumulative gains (Table 2), and during weeks 4 and 5 higher feed consumptions (Table 4) in comparison to the control treatment (red). The other treatments were intermediate. Weekly weight gains for rats receiving the blue treatment appeared higher, but this difference was not significant (Table 3). Similarly, feed conversions were not different (Figure 3).

The micelle treatment in saline (blue) resulted in a divergence of the growth curves starting at week 3 after first immunization (Table 2 and Figure 1). This is not surprising since it would be at this time that the major secondary antibody response to the booster injection would have occurred.

Antibody titers were measured in blood samples obtained at 4 weeks from all groups. Titers were very low. In fact titers were only barely detectable in about 10 rats (between 2 and 5% binding at 1:? dilution) randomly distributed over the 4 immunization treatments.

The lack of antibody titer development is difficult to explain, especially in light of the increased growth of the rats receiving the blue treatment. However, there is a precedent in the literature. Carelli et al. at the Pasteur Institute, Paris immunized mice against gonadotropin releasing hormone to induce immunological castration. The immunization was physiologically very successful. Spermatogenesis was clearly reduced an mostly absent. However, antibody titers were also extremely low, in fact 16% binding at dilutions of 1:2 to 1:4. This titer is similar to the one we observed in rats.

It is possible that measurement of low titers is an artifact of the technique used. In fact, the presence of free antibodies is measured only.

Measurement of a high titer may present misleading information, since it does not consider the quality or affinity of the antibodies. Thus a high titer could mean an abundance of low affinity antibodies which show relatively low immunoneutralization of somatostatin. This would result in little reduction in free somatostatin levels in plasma. Low titers as measured in this experiment could indicate the presence of high affinity antibodies with high immuno-neutralization of somatostatin and a substantial reduction in free somatostatin levels in plasma. We are presently developing a technique to measure free somatostatin in plasma after removal of immunoglobulins.

The results of this study should be interpreted carefully, since the number of animals used per treatment was low. However, since significant changes were observed, we conclude the following:

1. Somatostatin-immunization has clear potential in enhancing growth, even in fast growing species such as the rat. The increase in growth was 12%.

2. The use of dimyristoyl-lysine-somatostatin derivative has potential application in the immunization technique, especially through the use of micelles or liposomes.

3. Further study is required to measure physiological changes in free somatostatin levels as it relates to the presence of antibodies and efficacy of immunoneutralization.

4. Ongoing studies are justified to further develop this technique of immunization.

Acknowledgements:

Funding for these studies was provided through SANF, ADF and ERDA.

Table 1. Absolute weights (grams) of rats immunized against somatostatin (SRIF-16)[1].

Week	Control n=6	Blue n=4	Orange n=5	Green n=5	Yellow n=5	SEM
0 (start)	139.6	140.3	140.0	138.9	140.6	8.9
1	189.4	194.5	191.9	188.9	192.7	8.9
2	240.2	246.7	246.0	237.2	242.7	11.0
3	283.9	295.5	292.5	280.8	289.4	9.6
4	321.9	339.0	331.3	321.3	327.8	11.6
5	350.8	374.6	363.8	352.1	356.3	13.7
6	374.4^{a}[2]	403.0^{b}	390.9^{ab}	378.5^{ab}	381.3^{ab}	14.5

[1] Rats were immunized at week 0 and 14 days later (week 2)

[2] Means with different superscripts are different; ($P<0.05$)

Table 2. Cumulative gain (grams) of rats immunized against somatostatin (SRIF 16).

Week	Control n=6	Blue n=4	Orange n=5	Green n=5	Yellow n=5	SEM
1	50.3	54.5	51.9	50.0	52.1	2.8
2	100.4	106.3	106.0	98.4	102.1	5.4
3	144.4^{ab}	155.2^{a}	152.5^{ab}	142.0^{b}	148.8^{ab}	6.8
4	182.4	198.7	191.3	182.4	187.2	10.3
5	211.2^{*2}	234.3^{*}	223.8	213.3	215.7	11.9
6	234.9^{a}	262.6^{b}	250.9^{ab}	239.6^{ab}	240.8^{ab}	12.9

1 Means with different superscripts are different; $P<0.05$.

2 $P<0.10$.

Table 3. Weekly weight gains (grams) of rats immunized against somatostatin (SRIF-16).

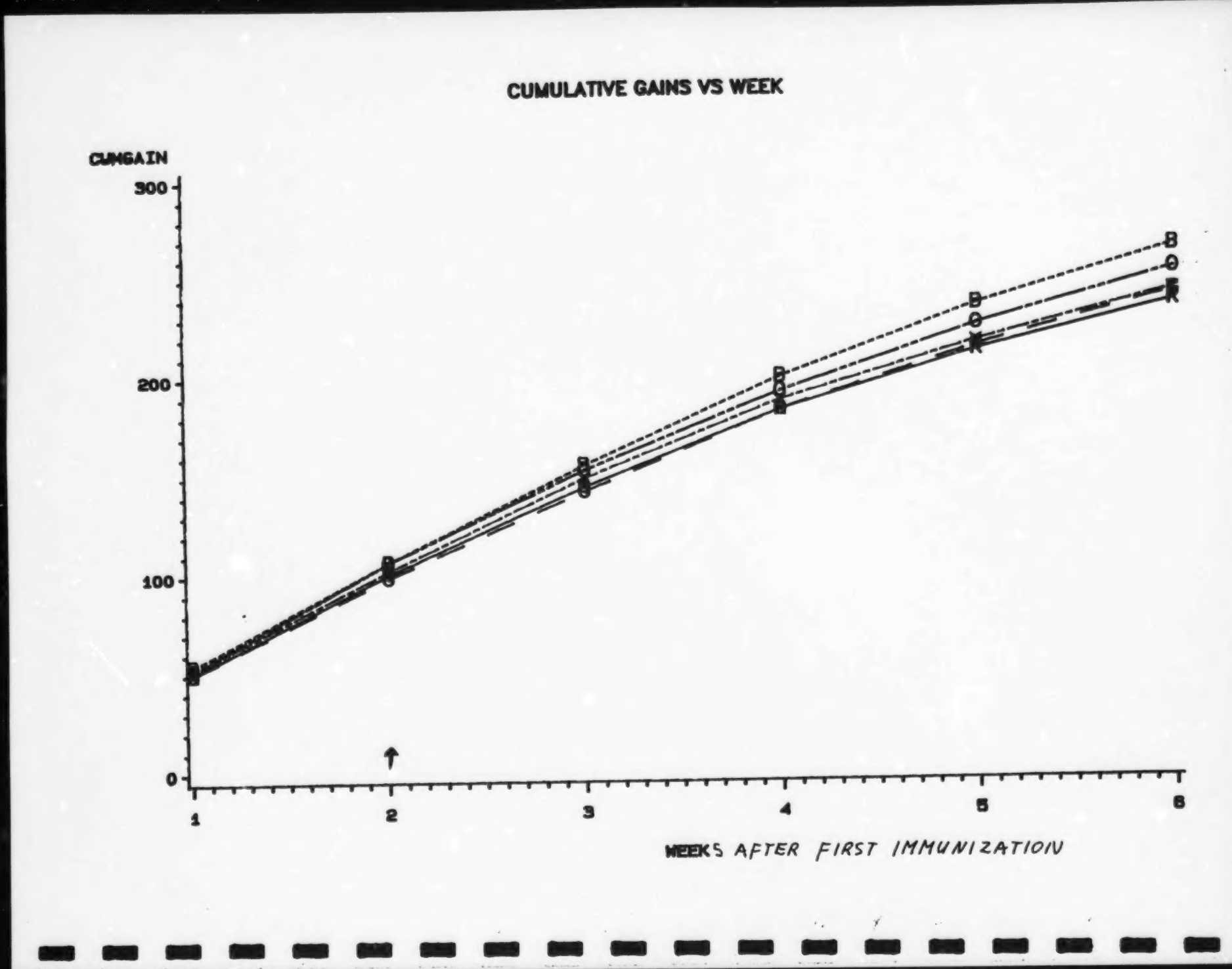
Week	Control n=6	Blue n=4	Orange n=5	Green n=5	Yellow n=5	SEM
1	49.8	54.2	51.9	50.0	52.1	2.8
2	50.9	52.2	54.1	48.4	50.0	4.6
3	43.7	48.9	46.6	43.6	46.7	4.6
4	38.0	43.5	38.7	40.5	38.4	4.7
5	28.9	35.6	32.5	30.8	28.5	4.6
6	23.6	28.4	27.1	26.4	25.0	4.0

Table 4. Weekly feed consumption (grams) of rats immunized against somatostatin (SRIF-16).

Week	Control n=6	Blue n=4	Orange n=5	Green n=5	Yellow n=5	SEM
1	153.2	161.4	156.6	153.7	159.1	10.3
2	168.1	175.5	179.6	169.2	173.9	11.0
3	172.6	184.8	181.2	170.4	180.7	8.4[2]
4	170.2[a1]	190.0[b]	183.4[ab]	179.0[ab]	179.3[ab]	8.9
5	172.04[a]	191.6[b]	184.4[ab]	180.0[ab]	181.8[ab]	9.7
6	183.0	198.3	195.6	189.9	192.4	8.9

[1] Means with different superscripts are different; $P<0.05$.

[2] $P<.10$.


CUMULATIVE GAINS VS WEEK
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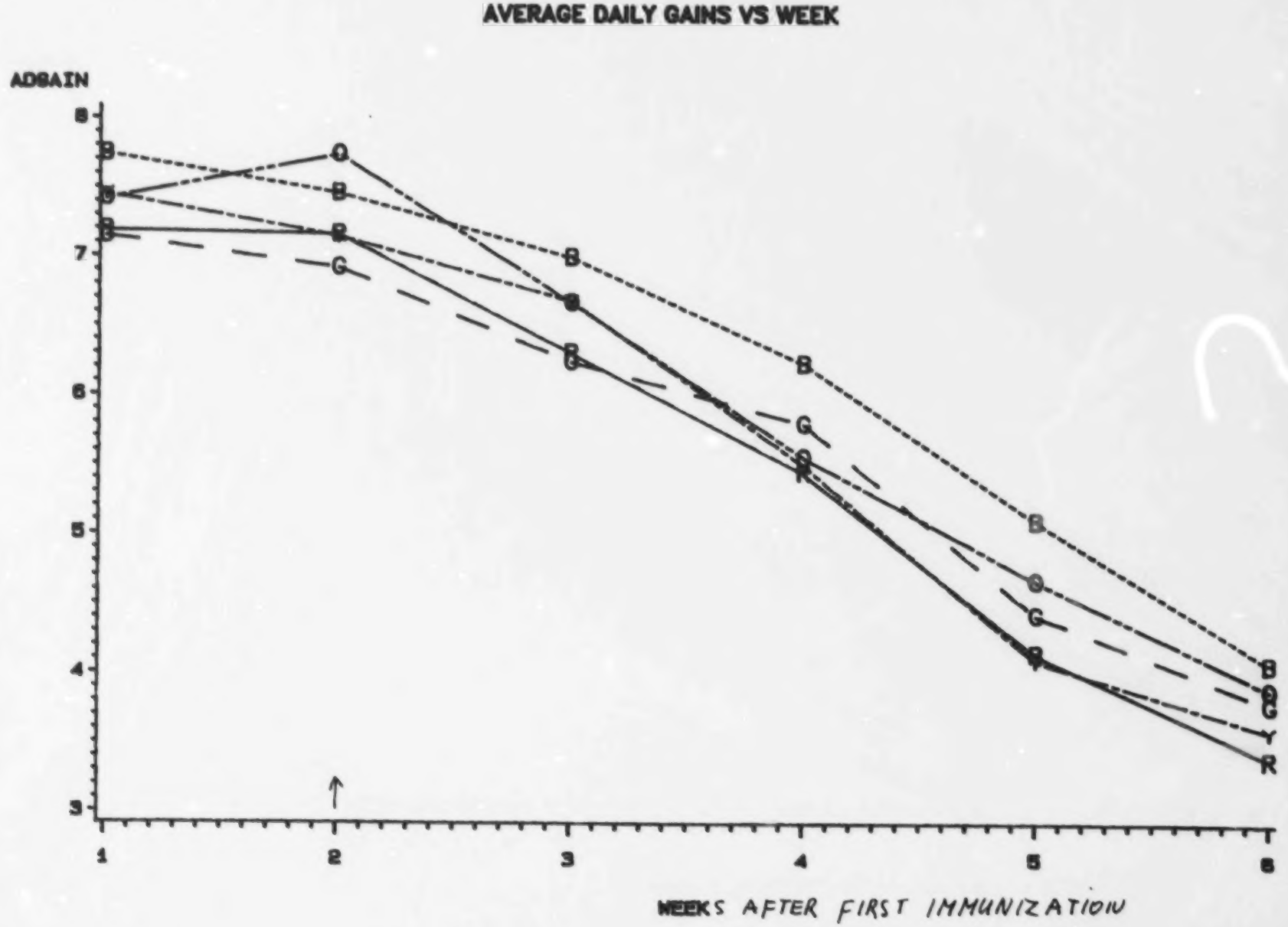
WEEKS AFTER FIRST IMMUNIZATION


AVERAGE DAILY GAINS VS WEEK
ADGAIN
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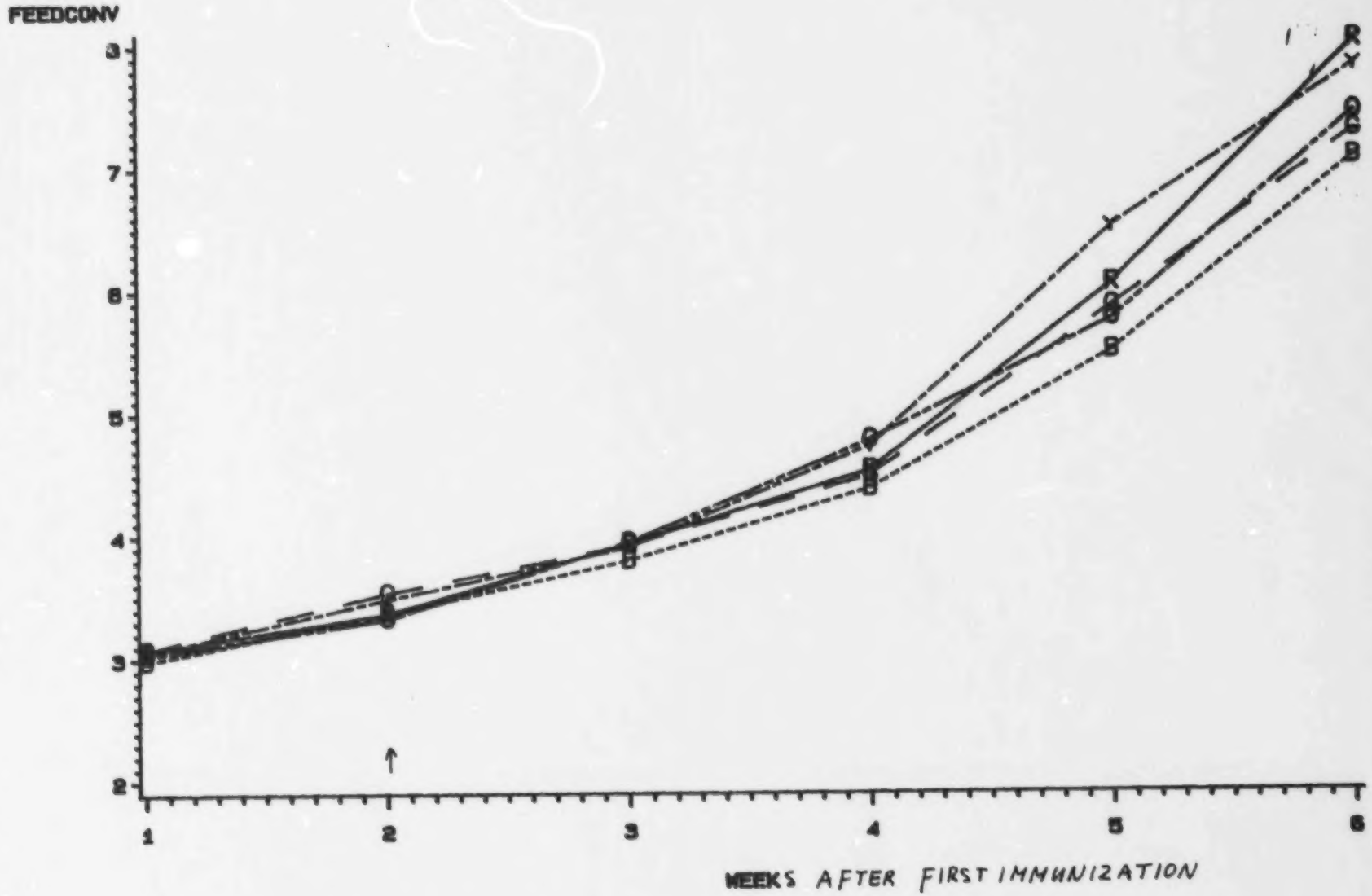
WEEKS AFTER FIRST IMMUNIZATION

FEED CONVERSIONS VS WEEK



FINAL REPORT PROJECT 5-85-26 SARF

~~5-85-01-015~~
~~5-86-01-007~~ March 30, 1987

THE IMPROVEMENT OF PRODUCTION EFFICIENCY OF LIVESTOCK BY VACCINATION AGAINST SOMATOSTATIN.

BACKGROUND AND OBJECTIVES

Somatostatin is a peptide hormone consisting of 14 amino acids. It is secreted from the hypothalamus and has been shown to strongly inhibit growth hormone secretion from the pituitary gland. It is desirable to increase the rate of secretion of growth hormone from the pituitary gland because higher levels of growth hormone in the blood result in higher average daily gains, leaner carcasses, better feed efficiency and increased milk production.

The objective of our on-going research is to develop a method which will neutralize somatostatin. This can be achieved by immunizing the animal against it own circulating somatostatin. The antibodies thus formed will neutralize the endogenous somatostatin, leading to increased levels of growth hormone in the blood.

The immunization technique consists of injecting a somatostatin-ovalbumin conjugate in the presence of an adjuvant. The purpose of the adjuvant is to illicit a strong immune response since the conjugate itself is only weakly immunogenic. The objective of the immunization technique is to achieve a quick and vigorous immune response. In fact, the immune response required to achieve growth promotion is considerably higher than that required for disease protection. Thus the choice of adjuvant is very important.

The purpose of the studies reported herein was to evaluate several commercially available adjuvants in somatostatin immunization in swine, sheep and rats.

EXPERIMENTAL METHODS

A sufficiently large batch of somatostatin-ovalbumin conjugate was prepared for immunization of pigs, lambs and rats. The procedure was as follows: Ovalbumin (36 mg; 8.5 mg/ml) was mixed with carbodiimide (EDC) (71.8 mg; 38.8 mg/ml). Somatostatin (9.8 mg; 4.3 mg/ml) was added dropwise while stirring. All solutions were in distilled water. The pH was adjusted to 5.5 and the reaction continued for 24 h at room temperature. The mixture was then dialyzed against several changes of distilled water for 12 h. Molecular cut off of the dialysis tubing was 8000. Efficiency of incorporation of somatostatin was 58% and the estimated molar ccupling ratio of somatostatin to ovalbumin was 3:1.

Swine experiment

Twenty 30 kg-piglets at the University of Saskatchewan feeder barn were assigned to five treatments (n=4; 2 male and 2 female pigs per pen).

Adjuvant Treatments were:

a. Control: ovalbumin carrier only (200 µg) in Havlogen.
b. Havlogen (10%) (HV).
c. Freund's Complete Adjuvant followed by Incomplete Adjuvant (FCA).
d. RIBI (Trehalose Dimycolate and Monophosphoryl Lipid A).
e. Regressin (BCG cell wall).

Treatments b. through e. consisted of the same dose of somatostatin-ovalbumin conjugate (50 µg somatostatin equivalent) with different commercial adjuvants. All animals were injected twice, fourteen days apart. Blood samples were obtained at 24 and 50 days after the first injection.

The study was not designed to evaluate growth performance, but growth and slaughter data were routinely collected. Pigs immunized with FCA were routinely collected. Pigs immunized with FCA were not commercially slaughtered. Treatment with FCA results in a false positive tuberculin test.

Lambs experiment

Forty-two lambs at the University of Saskatchewan sheep unit at weaning were assigned to three adjuvant treatments (n=14).

a. RIBI.
b. Havlogen (10%).
c. Control (ovalbumin carrier in Havlogen).

Lambs assigned to treatments a. and b. received 100 µg somatostatin equivalent per injection. Lambs were injected twice, 14 days apart. Blood samples were obtained weekly and weight gains were monitored.

Rat experiment

Rats were assigned to four adjuvant treatments (n=15; 7 male-8 female)

a. Control (ovalbumin carrier (20 µg) in Havlogen).
b. RIBI.
c. Havlogen (10%).
d. Havlogen with Regressin.

Rats assigned to treatments b. through d. received 5 µg somatostatin equivalent per injection. Two injections were given 14 days apart.

In all studies anti-somatostatin antibodies in plasma were measured by incubating serially diluted plasma with 5000 cpm of radioactive somatostatin. The titer reported indicates the dilution of plasma required to obtain 50% binding of the radioactive somatostatin in a final buffer volume of 0.7 ml.

RESULTS AND DISCUSSION

Swine experiment

The antibody titers against somatostatin present at 24 and 50 days after the first injection are presented in Table 1. No antibodies were detectable in pigs receiving the control treatment. Freund's Complete Adjuvant (FCA)

induced a very strong immune response, whereas the other adjuvants were very weak. It is desirable to achieve a titer of at least 1:1000. Thus the commercial adjuvants were ineffective with respect to somatostatin-immunization. The high titers in the FCA treatment confirm that the lack of immune response in the other treatments is the result of low adjuvanticity. However, the FCA treatment can not be used in commercial animals. The affinity of the antisera generated by the four pigs treated with FCA was measured by Scatchard plot at a serum dilution giving 50% binding. The FCA treatment induced antisera of high affinity (Kd= 3.56 pM) which is one to two orders of magnitude higher than the affinity of the receptor for somatostatin.

The growth performance of the pigs is indicated in Table 2. The FCA treatment resulted in a lower growth performance, despite the high antibody titers. This is not surprising since these pigs developed severe abscesses at the site of injection, probably causing considerable stress to the animal. An additional criterion for evaluation of an adjuvant would be the absence of stress to the animal and interference with growth performance.

Lamb experiment

Antibody levels in lambs immunized with the adjuvants Havlogen and RIBI were low: <50% binding at 1:7 dilution (Table 3). However, the fairly low antibody levels did persist until completion of the study. The Havlogen treatment appeared to induce slightly higher antibody levels than the RIBI adjuvant. It is questionable whether the antibody levels achieved were high enough to permanently affect growth performance in lambs. Havlogen treated lambs showed a heavier final weight and higher (10%) average daily gains (Table 4), but these differences were not significant ($P>0.05$). It is possible that with a larger number of animals the difference would have been statistically significantly different. Havlogen immunized lambs did show a higher ($P<0.05$) rate of gain during the last two weeks of the study. In fact, this higher rate of gain accounted for most of the difference in final body weight between Havlogen treated and control lambs.

The RIBI adjuvant treatment did not result in improved antibody levels and growth performance of the lambs was almost identical to that of the control lambs.

Rat experiment

The antibody titers generated in the rat experiment were very similar to those in the lamb experiment (Table 5). Havlogen did appear to be a better adjuvant than RIBI. However, the levels of antibody titers achieved were not satisfactory.

Growth performance of the rats did not appear to be affected by the treatments. However, there was a major sex difference. Overall it appeared that the treatments were not effective since a high level of antibodies was not achieved.

Table 1. Somatostatin antibody titers[1] of pigs immunized with a somatostatin-ovalbumin conjugate with different adjuvants

	Control	Havlogen	FCA[2]	Ribi	Regressin
24 days post-injection					
pig 1	ND3	4	4000	ND	4
pig 2	ND	4	2500	ND	6
pig 3	ND	ND	2500	ND	3
pig 4	ND	7	12500	ND	2
59 days post-injection					
pig 1	ND	ND	2000	2	ND
pig 2	ND	ND	1000	ND	2
pig 3	ND	ND	3000	2	ND
pig 4	ND	4	7000	2	ND

1 Dilution of plasma required to achieve 50% binding of 5000 cpm of radioactive somatostatin in a final vlume of 0.7 ml.

2 Freunds Complete Adjuvant.

3 Not detectable.

Table 2. Performance of pigs immunized against somatostatin

	Control	HV	FCA	RIBI	Regressin
Average daily gain, kg	0.82	0.86	0.74	0.72	0.78
Feed intake, kg/day	2.43	2.40	2.29	2.21	2.14
Feed conversion	2.96	2.77	3.08	3.07	2.74
Final Weight, kg	99.50	97.88	N.A.*	95.50	98.50
Carcass weight, kg	77.35	74.65	N.A.	74.60	77.10
Dressing %	77.70	76.30	N.A.	78.10	78.20
Index	102.25	104.25	N.A.	106.25	105.25
Fat, inches	1.45	1.28	N.A.	1.05	1.23

* Not available

Table 3. Antibody titers[1] in lambs immunized twice two weeks apart with somatostatin-ovalbumin conjugate and different adjuvants

	Control	Havlogen	RIBI
Weeks after primary immunization[2]			
2	0	0	0
4	0	37 ± 4.5[3]	12.6 ± 2.4
5	0	30.2 ± 4.5	9.9 ± 2.2
6	0	38.2 ± 3.8	15.8 ± 1.7
7	0	35.0 ± 4.0	23.3 ± 1.2

1 % binding of 5000 cpm somatostatin at a final dilution of 1:7; final volume 0.7 ml.

2 A booster injection was given two weeks after primary immunization.

3 Mean SE.

Table 4. Growth of lambs immunized against somatostatin using two different adjuvants.

	Control	Havlogen	RIBI
Birthweight, kg	4.45 ± 0.3	4.51 ± 0.3	4.74 ± 0.26
Weaning weight, kg	15.4 ± 1.0	15.4 ± 1.0	15.9 ± 0.9
Start of test, kg	18.3 ± 1.2	18.4 ± 1.2	18.3 ± 1.1
Final weight, kg	36.3 ± 1.6	38.2 ± 1.6	36.7 ± 2.1
Average daily gain on test, g/day	367 ± 17	404 ± 15	376 ± 24

Table 5. Antibody response[1] and growth performance of rats immunized against somatostatin using different adjuvants.

	Control		Havlogen		RIBI	
Final titer[2]	0		41.3 ± 6.2		13.5 ± 4.7	
% responders	-		86		62	
	M[3]	F	M	F	M	F
Starting weight, g	81.8±2.7[4]	78.3±2.2	84.7±2.1	78.9±1.7	80.9±2.3	79.0±1.9
Final weight, g	319 ±8.5	206.4±6.2	324.4±7.5	204.9±4.1	332.5±6.1	205.2±4.7
Daily gain, g	6.8±0.2	3.7±0.2	6.9±0.2	3.6±0.1	7.2±0.1	3.6±0.1
Feed conversion[5]	3.75±0.05	5.09±0.14	3.60±0.06	5.13±0.07	3.52±0.03	5.22±0.03

1 % binding of 5000 cpm somatostatin at a final dilution of 1:10; final volume 0.7 ml.

2 %.

3 M=male, F=female.

4 Mean SE.

5 Grams of feed per gram of gain.

PAPERS AND PRESENTATIONS:

FORMAL:

B. Laarveld, R.K. Chaplin and D.E. Kerr, 1986. Somatostatin immunization and growth of lambs. Canadian Journal of Animal Science 66:77-83.

B. Laarveld, 1986. Potential of immunization against somatostatin as a method of growth promotion. Eli-Lilly Research Laboratories, Greenfield, Indiana; June 3.

B. Laarveld, 1986. Modulation of growth in ruminants through hormonal manipulation. Nutrition Day Conference, Macdonald College of McGill University, Ste. Anne de Bellevue, Que; April 2.

B. Laarveld, 1987. Growth hormones. Saskatchewan Provincial Dairy Convention, Regina Inn, Regina; February 11.

B. Laarveld, 1986. Saskatchewan DHAS supervisor meeting, Circle 8 Building, Saskatoon. Somatotropin in dairy production; 90 minute lecture; October 2.

RADIO OR TELEVISION:

September 8, 1986, CJWW radio; Growth hormones.

February 10, 1987, CJWW radio; Somatotropin for dairy cows.

March 2, 1987, CBC television 'Countryside'; Somatotropin and stimulation of milk production in dairy cows.

INFORMAL PRESENTATIONS:

Several presentations to R.O.P. Committees, SDA extension specialists, Chinese Trade Delegations (2) on new techniques of promoting growth in farm animals and new methods of selecting genetically superior animals.

PERSONNEL INVOLVED IN THE PROJECT:

Mrs. Hampton, Research Technician	80%
Mr. Korchinski, Animal Technician	80%
Ms. J. Hamilton, Research Assistant	5%
Mr. A. van Kessel, M.Sc. student	100%
Dr. P.A. Thacker, Assistant Professor	5%
Dr. R.K. Chaplin, Associate Professor	10%
Dr. B. Laarveld, Associate Professor	30%

SUMMARY

Somatostatin is a peptide hormone consisting of 14 amino acids. It is secreted from the hypothalamus and has been shown to strongly inhibit growth hormone secretion from the pituitary gland. It is desirable to increase the rate of secretion of growth hormone from the pituitary gland because higher levels of growth hormone in the blood result in higher average daily gains, leaner carcasses, better feed efficiency and increased milk production.

The objective of our on-going research is to develop a method which will neutralize somatostatin. This can be achieved by immunizing the animal against its own circulating somatostatin, leading to increased levels of growth hormone in the blood.

A major limitation to the development of the technique is that animals do not produce antibodies against their own hormones. Therefore somatostatin is chemically coupled to a foreign protein such as ovalbumin or egg white. Upon immunization the animal then will mount an immune response to both ovalbumin and somatostatin. The appropriate procedure for coupling the peptide is under development. A second limitation concerns the magnitude of the immune response required. For prevention of disease only low levels of antibodies after vaccination are needed. However for neutralization of somatostatin considerably larger amounts of antibodies are necessary. The immune response normally is stimulated by including an adjuvant in the vaccine. We have evaluated various commercially available adjuvants in lambs, pigs and rats and conclude that none are satisfactory for immunization against somatostatin. We are continuing our studies in this regard. Development of a method of immunization against somatostatin is desirable. Somatostatin immunization has application in all species of farm animals and provides a cheap, safe method of promotion of growth and lactation.

ABSTRACT

Immunization of farm animals against somatostatin has potential as a method for promoting growth and lactation. Somatostatin is a hormone which strongly inhibits secretion from the pituitary gland. Thus, removal of somatostatin through immunization results in higher blood growth hormone levels. A series of studies determined that the immune response to the somatostatin-ovalbumin vaccine is weak. Commercially available adjuvants such as Alhydrogel, Havlogen, Ribi, Regressin do not have sufficient adjuvanticity to induce a strong immune response. Studies are in progress to identify or develop an adjuvant which will serve both as a carrier and as an immunogenic stimulator.